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Michelle Hobson

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

E. Rebar *et al.*

Application No.: 10/055,711

Filed: January 22, 2002

For: MODIFIED ZINC FINGER BINDING PROTEINS

Examiner: Jennifer A. Dunston

Group Art Unit: 1636

Confirmation No.: 6236

REPLY BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Reply Brief is filed in response to an Examiner's Answer mailed January 4, 2010, making a Reply Brief due on or before March 4, 2010. Accordingly, this Reply Brief is timely filed.

REAL PARTY IN INTEREST

Sangamo BioSciences, Inc. is the assignee of record in this case by virtue of an assignment recorded on April 24, 2002 at Reel 012622, Frame 0897. Thus, Sangamo BioSciences, Inc. is the real party in interest.

RELATED APPEALS AND INTERFERENCES

Appellants noted in their Appeal Brief that an Appeal Brief was filed on November 27, 2006 in U.S. Serial No. 10/470,180 (now U.S. Patent No. 7,262,054).

In the Examiner's Answer, it was noted that the facts in that case are not the same as the instant case. (Examiner's Answer, page 2). Accordingly, the Examiner did not consider the Appeal in U.S. Serial No. 10/470,180 to be related to the instant appeal. *Id.*

STATUS OF THE CLAIMS

Pending: claims 1, 23-28, 30-48 and 52-57

Withdrawn: 1, 23, 24, 33-35, 38, 42-48 and 52

Canceled: claims 2-22, 29, 49-51 and 58-61

Rejected: claims 25-28, 30-32, 36, 37, 39-41 and 53-57

Appealed: claims 25-28, 30-32, 36, 37, 39-41 and 53-57

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether claims 25-28, 30-32, 36-37, 39-41 and 53-57 are unpatentable under 35 U.S.C. § 103(a) in view of U.S. Patent No. 7,151,201 (hereinafter "Barbas '201") in view of Filippova (1996) *Mol. Cell Biol.* 16(6):2802-2813 (hereinafter "Filippova").

B. Whether claims 25-28, 30-32, 36, 39-41 and 53-57 are unpatentable under 35 U.S.C. § 103(a) in view of U.S. Patent No. 7,329,728 (hereinafter "Barbas '728") in view of Filippova.

C. Whether claim 37 is unpatentable under 35 U.S.C. § 103(a) over Barbas '728 in view of Filippova and further in view of Guyer et al. (1998) *Genetics* 149:633-639 (hereinafter "Guyer").

ARGUMENTS

A. The claims are non-obvious over Barbas '201 and Filippova

Claims 25-28, 30-32, 36-37, 39-41 and 53-57 remained rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over U.S. Patent No. 7,151,201 (hereinafter "Barbas '201") in view of Filippova (1996) *Mol. Cell Biol.* 16(6):2802-2813 (hereinafter "Filippova"). (Examiner's Answer, pages 4-7). Barbas '201 was again cited for allegedly teaching alteration and combining of any zinc finger protein frameworks and Filippova was cited for teaching the human CTCF protein, in which the 11th zinc finger has a CCHC structure. *Id.* It was alleged that it would have been obvious to take Filippova's 11th finger, modify its recognition helix in the context of other zinc fingers. *Id.*

In response to Appellants' arguments that Barbas '201 and Filippova do not teach or suggest all the elements of the claims, it was asserted that "all of the structural and functional elements are met by the combined teachings of Barbas and Filippova." (Examiner's Answer, page 14). Furthermore, it was also alleged that Barbas's teachings regarding frameworks include joining different zinc finger frameworks into one protein made up of a single finger isolated from its natural context (Examiner's Answer, page 16):

> According to the teachings of Barbas, III et al. it would have been within the skill of the art to use a zinc finger framework that includes a single zinc finger from any known protein, to mutagenize the zinc finger domain within the recognition helix and to combine the zinc finger domain with other zinc finger domains (e.g., column 1, line 53 to col 2, line 6; column 3, lines 36-43; column 4, line 59 to column 5, line 11; column 18, line 47 to column 20, line 40).

See, also, page 18 of the Examiner's Answer for the same citations to Barbas '201 regarding this reference's alleged teachings regarding combining different zinc finger domains. In addition, it was again alleged that Filippova's zinc finger meets Barbas 201's definition of a "zinc finger." (Examiner's Answer, pages 21-22). Various references of record were also cited on page 20 of the Examiner's Answer regarding engineering of the recognition helix region to bind to a target site.

In response to Appellants arguments that Filippova teaches away from isolating the 11th finger of CTCF for DNA-binding, it was alleged that Filippova teach that this finger is involved binding and does not criticize the use of this framework in isolation (Examiner's Answer, page 22-24):

> These arguments are not persuasive. Filippova teach that finger 11 is involved in specifically binding to the P2-proximal site A of the human c-myc gene (e.g., page 2807, right column, 2nd full paragraph; Figure 7). Filippova does not criticize, discredit or otherwise discourage the use of CTCF zinc finger domains as frameworks in engineered zinc finger proteins. ...
>
> Even though the full-length, naturally occurring CTCF proteins uses different combinations of zinc fingers to bind to target sites, finger 11 is known to bind to a target DNA sequence, and it would have been within the skill of the art to use CTCF zinc finger domain(s) as a framework to engineer zinc finger proteins that bind to contiguous sequences according to the teachings of Barbas.

With regard to Appellants' arguments that it was not predictable to take Filippova's 11th finger out of its natural context, modify its recognition helix and put it in the context of other zinc finger frameworks, it was asserted that because removal of finger 11 abolishes binding of CTCF to binding site A, it would have been predictable to take this finger out of context and that it would retain its essential function after its recognition helix was altered in the context of different zinc fingers. (Examiner's Answer, page 25). The Examiner also asserted that Appellants statements on page 8-9 of their Brief somehow acknowledged that it was predictable to modify the recognition helix of any zinc finger protein at the time of filing, regardless of framework. (Examiner's Answer, page 27).

Appellants address the errors in the Examiner's assertions in turn.

a. Barbas '201 and Filippova do not the claimed elements

As noted in the Appeal Brief, in order to establish obviousness of a claimed invention, all the features of the claims must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Thus, in the pending case, the

combination of references must disclose a polynucleotide that encodes a non-naturally occurring zinc finger protein that includes a non-canonical (non-Cys2His2) zinc finger with the recited number of amino acids between zinc coordinating residues and in which the recognition helix of the non-canonical zinc finger has been altered to bind to a target site in a plant gene.

In the case on appeal, it is acknowledged that Filippova fails to teach anything about modifying the recognition helices of zinc fingers as claimed, anything about binding of a non-canonical zinc finger to a gene in a plant cell or anything about isolating a single finger (finger 11) from the context of the surrounding finger(s). Thus, these elements must be supplied by Barbas '201.

For the reasons of record and reiterated herein, the Examiner errs in relying on Barbas '201 as teaching the claimed elements not taught by Filippova. Most egregiously, the Examiner errs in alleging that Barbas somehow teaches combining different "frameworks" to form a single zinc finger protein. In support this position, the Examiner cites the following passages of Barbas '201 (column 1, line 53 to col 2, line 6; column 3, lines 36-43; column 4, line 59 to column 5, line 11; column 18, line 47 to column 20, line 40 to column 25, line 8, emphasis added):

> In the context of regulatory transcription factors, zinc finger domains which are responsible for specifically targeting a particular nucleotide sequence within a gene are generally coupled to additional amino acid sequences which serve to modulate expression either by activating (amplifying) or repressing it. Thus, typical transcription regulatory factors comprise both **a** zinc finger domain responsible for targeting the appropriate position of the genome and a functional portion which controls transcription of the gene once the fusion protein is bound.
>
> Synthetic zinc finger proteins have been synthesized and found to have binding affinity similar to those found in native transcription factors. Further, zinc finger proteins have been designed which are specific for TGA or for one of the triplets of the formula GNN. Thus, zinc finger proteins can be designed to target unique sequences of the formula (GNN).sub.6 or sequences containing 18 nucleotides wherein some of the GNN triplets have been substituted by TGA. As the design of zinc finger

proteins progresses, appropriate zinc finger domains can be designed for any desired target sequence.

More preferably, the target nucleotide sequence comprises 18 nucleotides and wherein the zinc finger protein is a hexadactyl zinc finger protein. Further preferably, the targeted nucleotide sequence is of the formula (GNN)n, and wherein N is any one of the A, T, C or G and n is an integer from 1 to 6. More preferably, the targeted nucleotide sequence is of the formula $(GNN)_6$, and wherein N is any one of the A, T, C or G.

The zinc finger protein used in the present methods can comprise a plurality of finger regions. The zinc finger protein can comprise linker regions among the plurality of finger regions. For example, **the** zinc finger protein used in the present method can contain any number of **the** 3-finger region. Preferably, the zinc finger protein can comprise at least two 3-finger regions that are separated and linked together with a linker region. The linker region can be any suitable length, e.g., from about 2 to about 10 amino acid residues in length. Preferably, the linker region between any said two 3-finger region is about 5 amino acid residues in length.

In addition to the typical zinc finger domains, the zinc finger protein can further comprise other desirable domains such as effector domains active in the host plant cells. Any types of zinc finger protein can be used in the present method. But preferably, the zinc finger protein comprising **a framework** from a plant zinc finger protein can be used. Alternatively, synthetic zinc finger proteins or non-naturally-occurring zinc finger proteins can be used.

Zinc finger proteins used in the current methods...

The zinc finger polypeptides used in the present method can be engineered to recognize a selected target site in the gene of choice. Typically, **a** backbone from any suitable C2H2-ZFP, such as SPA, SPIC, **or** ZIF268, is used as **the** scaffold for the engineered zinc finger polypeptides (see, e.g., Jacobs, EMBO J. (1992) 11:4507; and Desjarlais & Berg, Proc. Natl. Acad. Sci. USA (1993) 90:2256–2260). A number of methods can then be used to design and select a zinc finger polypeptide with high affinity for its target. A zinc finger polypeptide can be designed or selected to bind to any suitable target site in the target gene, with high affinity.

A useful zinc finger framework is that of ZIF268 (see WO00/23464 and references cited therein), however, **others** are suitable. Examples of known zinc finger nucleotide binding polypeptides that can be truncated, expanded, and/or mutagenized in order to change the function of a

> nucleotide sequence containing a zinc finger nucleotide binding motif includes TFIIIA and zif268. Other zinc finger nucleotide binding proteins are known to those of skill in the art. The murine CYS2-HiS2 zinc finger protein Zif268 is structurally well characterized of the zinc finger proteins (Pavletich and Pabo, *Science* (1991) 252:809–817; Elrod-Erickson et al., *Structure* (*London*) (1996) 4:1171–1180; and Swirnoff et al., *Mol. Cell. Biol.* (1995) 15:2275–2287).
>
> In a specific embodiment, the zinc finger protein used in the present methods comprises **a** framework (or backbone) derived from **a** naturally occurring zinc finger protein. Framework (or backbone) derived from **any** naturally occurring zinc finger protein can be used. For example, the zinc finger protein comprises a framework (or backbone) derived from a zinc finger protein comprising a C2H2 motif can be used. Preferably, the protein or peptide sequence within the β sheet of the C2H2 motif is not substantially changed, or not changed, from its natural sequence.
>
> In another specific embodiment, the zinc finger protein used in the present methods comprises **a** framework (or backbone) derived from a zinc finger protein that is naturally functional in plant cells. For example, the zinc finger protein used in the present methods can comprise **a** C3H zinc finger (Terol et al., Gene, 260(1–2):45–53 (2000)), a QALGGH motif (Takatsuji, Plant. Mol. Biol., 39(6):1073–8 (1999)), a RING-H2 zinc finger motif (Jensen et al., FEBS Lett., 436(2):283–7 (1998)), a 9 amino acid C2H2 motif (Chou et al., Proc. Natl. Acad. Sci. USA, 95(9):5293–8 (1998)), a zinc finger motif of Arabidopsis LSD1 (Dietrich et al., Cell, 88(5):685–94 (1997)) and a zinc finger motif of BBF/Dof domain proteins (De Paolis et al., Plant J., 10(2):215–23 (1996)).

To the extent that these passages are even relevant to combining zinc finger frameworks from different zinc finger proteins, Barbas '201 teaches, at best, that 3-fingered frameworks of the same of backbone (C2H2) can be linked together to form longer proteins. These passages say nothing about isolating individual fingers from their natural context, let alone anything about mixing and matching these individual fingers to form a novel zinc finger protein.

Therefore, it remains the case that Barbas '201 does not teach or suggest isolating a single zinc finger from its natural context and then combining the isolated finger with different fingers from other proteins. In fact, with regard to linking of zinc finger proteins, Barbas '201 relates solely to linkage of multi-finger proteins (Barbas '201, col.

4, line 59 to col. 5, line 2, also reproduced above; col. 20, lines 41-45; col. 58, lines 9-13 and 18-20; col. 59, lines 36-37, emphasis added):

> The zinc finger protein used in the present methods can comprise a plurality of finger regions. The zinc finger protein can comprise linker regions among the plurality of finger regions. For example, the zinc finger protein used in the present method can contain any number of the 3-finger region. Preferably, the zinc finger protein can comprise at least two 3-finger regions that are separated and linked together with a linker region. The linker region can be any suitable length, e.g., from about 2 to about 10 amino acid residues in length. Preferably, the linker region between any said two 3-finger region is about 5 amino acid residues in length.
>
> The amino acid linker should be flexible, a beta turn structure is preferred, to allow **each three finger domain** to independently bind to its target sequence and avoid steric hindrance of each other's binding. Linkers can be designed and empirically tested.
>
> **A** human zinc finger protein Sp1C has been selected to serve as a framework in the present example. It has been demonstrated that the Sp1C protein can provide a good framework for zinc finger modification.
> ...
>
> These **three-finger** ZFPs are then fused together to create a six-finger (polydactyl) zinc finger protein to bind a specific 18 bp sequence.

Thus, Barbas '201 fails utterly to disclose that a framework can be **a** single finger taken out of its natural context and combined with other fingers from different frameworks. In all cases, Barbas '201 suggests at best that **one** naturally occurring multi-finger zinc protein can be modified in the recognition helix region (Barbas '201, column 20, lines 3-10; column 21, lines 8 to 20; column 22, lines 51-60; column 22, lines 61 to column 23, line 6l; column 23, lines 8-11; column 23, lines 12-15; column 23, lines 23-25; emphasis added)

> As used herein, "framework (or backbone) derived from **a** naturally occurring zinc finger protein" means that the protein or peptide sequence within the naturally occurring zinc finger protein that is involved in non-sequence specific binding with a target nucleotide sequence is **not** substantially changed from its natural sequence. ....

The zinc finger polypeptides used in the present method can be engineered to recognize a selected target site in the gene of choice. Typically, **a** backbone from any suitable C2H2-ZFP, such as SPA, SPIC, or ZIF268, is used as the scaffold for the engineered zinc finger polypeptides (see, e.g., Jacobs, EMBO J. (1992) 11:4507; and Desjarlais & Berg, Proc. Natl. Acad. Sci. USA (1993) 90:2256–2260). A number of methods can then be used to design and select a zinc finger polypeptide with high affinity for its target. A zinc finger polypeptide can be designed or selected to bind to any suitable target site in the target gene, with high affinity.

A useful zinc finger framework is that of ZIF268 (see WO00/23464 and references cited therein), however, others are suitable. Examples of known zinc finger nucleotide binding polypeptides that can be truncated, expanded, and/or mutagenized in order to change the function of a nucleotide sequence containing a zinc finger nucleotide binding motif includes TFIIIA and zif268. Other zinc finger nucleotide binding proteins are known to those of skill in the art. The murine CYS2-HiS2 zinc finger protein Zif268 is structurally well characterized of the zinc finger proteins (Pavletich and Pabo, *Science* (1991) 252:809–817; Elrod-Erickson et al., *Structure* (*London*) (1996) 4:1171–1180; and Swirnoff et al., *Mol. Cell. Biol.* (1995) 15:2275–2287).

In a specific embodiment, the zinc finger protein used in the present methods comprises **a** framework (or backbone) derived from a naturally occurring zinc finger protein. Framework (or backbone) derived from any naturally occurring zinc finger protein can be used. For example, the zinc finger protein comprises a framework (or backbone) derived from a zinc finger protein comprising a C2H2 motif can be used. Preferably, the protein or peptide sequence within the β sheet of the C2H2 motif is not substantially changed, or not changed, from its natural sequence.

In another specific embodiment, the zinc finger protein used in the present methods comprises **a** framework (or backbone) derived from a zinc finger protein that is naturally functional in plant cells. For example, the zinc finger protein used in the present methods can comprise **a** C3H zinc finger (Terol et al., Gene, 260(1–2):45–53 (2000)), a QALGGH motif (Takatsuji, Plant. Mol. Biol., 39(6):1073–8 (1999)), a RING-H2 zinc finger motif (Jensen et al., FEBS Lett., 436(2):283–7 (1998)), a 9 amino acid C2H2 motif (Chou et al., Proc. Natl. Acad. Sci. USA, 95(9):5293–8 (1998)), a zinc finger motif of Arabidopsis LSD1 (Dietrich et al., Cell, 88(5):685–94 (1997)) and a zinc finger motif of BBF/Dof domain proteins (De Paolis et al., Plant J., 10(2):215–23 (1996)).

> In another specific embodiment, the zinc finger protein used in the present methods comprises **a** framework (or backbone) derived from a zinc finger protein that is known in the art as of Jan. 19, 2001.
>
> For example, the zinc finger protein used in the present methods can comprise **a** framework (or backbone) derived from the zinc finger protein disclosed in the following U.S. patents and PCT patent publications: ...
>
> The zinc finger protein used in the present methods can also comprise **a** framework (or backbone) derived from the zinc finger protein disclosed in the following references: ...

Throughout the disclosure, Barbas '201 refers only to one multi-finger protein framework that is then modified in its recognition helix region and, optionally, combined with the same multi-finger framework to form dimers. The Examiner has failed to point to anything in Barbas '201 (or in Filippova) that teaches combining a single finger from one protein with one or more fingers from another framework protein. Thus, the basis on which the rejection is premised is faulty as the references do not teach or suggest using a single non-canonical zinc finger with a modified recognition helix region combined with one or more fingers from different zinc finger proteins.

(b) Barbas '201 does not enable combining zinc fingers from different "frameworks"

In addition to failing to teach the claimed elements, the references fail to enable the skilled artisan to isolate single fingers from their natural framework, alter their recognition helix and recombine them with fingers from other frameworks.

It is axiomatic that to be available as a reference under 35 U.S.C. § 103, the reference must contain an enabling disclosure with regard to the claimed subject matter. *See, e.g., Chester v. Miller,* 906 F.2d at 1576 n.2, 15 USPQ2d at 1336 n.2 (Fed. Cir. 1990); *Titanium Metals Corp. of America v. Banner,* 778 F.2d at 781, 227 USPQ at 778 (Fed. Cir. 1985); *Scripps Clinic & Research Found. v. Genentech, Inc.,* 927 F.2d 1565, 1578, 18 USPQ2d 1001, 1011 (Fed. Cir. 1991); *Helifix Ltd. v. Blok-Lok Ltd.,* 208 F.3d 1339, 54 USPQ2d 1299 (Fed. Cir. 2000). In other words, the reference must "sufficiently

describe the claimed invention to have placed the public in possession of it." See, *Minnesota Mining & Mfg. Co. ("3M") v. Johnson & Johnson Orthopaedics, Inc.*, 976 F.2d 1559, 1572, 24 USPQ2d 1321, 1332 (Fed. Cir. 1992); see also *In re Donohue,* 766 F.2d 531, 533, 226 USPQ 619, 621 (Fed. Cir. 1985). As set forth in *In re Kumar*, 418 F.3d 1361 (Fed. Cir. 2005), to "render a later invention unpatentable for obviousness, the prior art must enable a person of ordinary skill in the art to make and use the later invention.... Thus, the relevant inquiry is... whether [the earlier patent] enabled persons skilled in this art to produce" the later invention."

In the case on appeal, Filippova is admittedly silent as to any modification of the CTCF zinc finger protein. For its part, Barbas '201 does not enable any modifications to the frameworks, including combining different frameworks. In fact, the evidence of record (including Barbas's own patents) establishes that it was entirely unpredictable at the time of filing what effects modifications outside the recognition helix region would have. Indeed, as noted in the specification, such "combination" zinc fingers had not been proposed, let alone actually tested (*see,* as-filed specification, page 3, lines 13-21, emphasis added):

> **[0008]** To date, however, cellular studies using designed C2H2 ZFPs have utilized relatively few positions in the zinc finger as adjustable parameters to obtain optimal activity. In particular, studies to date have modified only those residues at the finger – DNA interface. These have included positions known to make direct base contacts, 'supporting' or 'buttressing' residues immediately adjacent to the base-contacting positions, and positions capable of contacting the phosphate backbone of the DNA. Furthermore, many observed effects have been quite modest, and the possibility that improved ZFP activities might be achieved via substitution of residues at other positions in the finger or using non-C2H2 polypeptides has remained completely uninvestigated.

There is no disclosure whatsoever in Barbas '201 regarding how to combine a single finger from one framework with finger(s) from another framework. As such, the skilled artisan would have no expectation that zinc finger proteins combining Filippova's 11th finger with Barbas '201's frameworks (*e.g.,* Sp1C) would be functional.

Indeed, Barbas '201 provides absolutely no guidance on how residues would be modified, let alone how to combine different frameworks. Again, the only residues Barbas '201 teaches how to modify are the amino acids within the DNA-binding recognition helix region (Barbas, col. 21, lines 7-39, emphasis added):

> A useful zinc finger framework is that of ZIF268 (see WO00/23464 and references cited therein), however, others are suitable. Examples of known zinc finger nucleotide binding polypeptides that can be truncated, expanded, and/or mutagenized in order to change the function of a nucleotide sequence containing a zinc finger nucleotide binding motif includes TFIIIA and zif268. Other zinc finger nucleotide binding proteins are known to those of skill in the art. The murine CYS2-HiS2 zinc finger protein Zif268 is structurally well characterized of the zinc finger proteins (Pavletich and Pabo, Science (1991) 252:809 817; Elrod-Erickson et al., Structure (London) (1996) 4:1171 1180; and Swirnoff et al., Mol. Cell. Biol. (1995) 15:2275 2287). DNA recognition in each of the three zinc finger domains of this protein is mediated by residues in the N-terminus of the alpha helix contacting primarily three nucleotides on a single strand of the DNA. The operator binding site for this three finger protein is 5'-GCGTGGGCG-'3. Structural studies of Zif268 and other related zinc finger-DNA complexes (Elrod-Erickson et al., Structure (London) (1998) 6:451 464; Kim and Berg, Nature Structural Biology (1996) 3:940 945; Pavletich and Pabo, Science (1993) 261:1701 1707; Houbaviy et al., Proc. Natl. Acad. Sci. USA (1996) 93:13577 13582; Fairall et al., Nature (London) (1993) 366:483 487; Wuttke et al., J. Mol. Biol. (1997) 273:183 206; Nolte et al., Proc. Natl. Acad. Sci. USA (1998) 95:2938 2943; and Narayan et al., J. Biol. Chem. (1997) 272:7801 7809) have shown that residues from primarily three positions on the α-helix, -1, 3, and 6, are involved in specific base contacts. Typically, the residue at position -1 of the α-helix contacts the 3' base of that finger's subsite while positions 3 and 6 contact the middle base and the 5' base, respectively.

Thus, it is error to assert that disclosures in the art regarding modifying the recognition helix region (as in Barbas '201 and in the various references cited on page 20 of the Examiner's Answer) in any way suggest combining a single non-canonical framework (the 11th finger of CTCF) into Barbas '201's polynucleotides, as alleged by the Examiner.

Given Barbas '201 teaches nothing about combining single fingers from different frameworks and, moreover, that such modifications were entirely unpredictable in their

effects, Barbas '201 clearly cannot enable the skilled artisan to make a polynucleotide as claimed based on any combination of Barbas '201 and Filippova.

(c) Filippova teaches away from isolating one finger from its natural context

It also remains error to assert that Filippova does not teach away from isolating the 11th finger of their CTCF and then using this single finger as a framework for binding to genes in plant cells. As noted above, the Examiner alleged that this reference somehow does not criticize the use of this framework in isolation (Examiner's Answer, page 22):

> These arguments are not persuasive. Filippova teach that finger 11 is involved in specifically binding to the P2-proximal site A of the human c-myc gene (e.g., page 2807, right column, 2nd full paragraph; Figure 7). Filippova does not criticize, discredit or otherwise discourage the use of CTCF zinc finger domains as frameworks in engineered zinc finger proteins. ...

As a threshold matter, Appellants note that it is not possible to show a negative – when the skilled artisan (as evidenced by Barbas and Filippova) did not even contemplate isolating a single finger "framework" and combining it with other protein "frameworks," it cannot be shown that it was tried, let alone that Filippova somehow criticizes or discredits this notion.

Furthermore, a teaching that finger 11 is sometimes (but not always) involved, along with other fingers in the naturally occurring CTCF protein, in binding to the human gene is not in any way a teaching or suggestion to isolate this single finger and re-combine it with other zinc fingers. Again, Filippova does not show that any fingers of CTCF can bind to plant genes and, with regard to finger 11, teaches that this finger is not involved in binding DNA in chickens (Filippova, Abstract):

> Although there is 100% sequence identity in the DNA binding domains of the avian and human CTCF proteins, the regulatory sequences recognized by CTCF in the chicken and human *c-myc* promoters are cleavage diverged. ... Gel shift assays utilizing successively deleted Zn finger domains indicate that CTCF Zn fingers 2 to 7 are involved in binding to

> the chicken *c-myc* promoter, while fingers 3-11 mediate CTCF binding to the human promoter.

Filippova teaches absolutely nothing about isolating one finger and suggests nothing about what this finger would do in isolation. Moreover, by virtue of the fact that Filippova is clear that finger 11 only binds to certain target sites in certain contexts, this reference does in fact discredit, discourage and/or criticize the proposed modification (isolation of a single finger of the CTCF framework) to this reference on which the rejection is based.

(d) The references and art as a whole teach away from combining different frameworks

The Examiner's assertion that Barbas teaches or suggests how to combine different frameworks is also completely contradicted by the evidence of record. As noted, additional publications by Barbas, namely for example U.S. Patent No. 7,329,729, evidence that the skilled artisan believed that modification of framework residues could impact DNA-binding. *See, e.g.,* Barbas '728 (discussed in detail below) which teaches that altered recognition helices function only in certain frameworks (Barbas '728 col. 42, lines 19-24):

> The framework residues play a role in affinity and specificity. Thus, amino acid positions −2 to 6 of the DNA recognition helices are either grafted into a Zif268 (Pavletich et al. (1991) Science 252:809-817) or an Sp1C framework (Desjarlais et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260).

Accordingly, the art teaches away from modifying these residues, instead teaching that only recognition helix residues of certain Cys2His2 frameworks were altered was binding function in any way predictable.

Likewise, Barbas '201 only teaches how to use a single (canonical) framework in which all the DNA binding zinc fingers are involved in binding. Filippova clearly teaches that the CTCF framework is a complicated protein in which the lone non-

canonical finger is not always involved in DNA binding functionality and teaches nothing about binding to plant genes.

As Barbas '201 and the art as a whole clearly establishes that the framework was considered relevant to binding, and teach away from modifications of the framework outside the recognition helix region, there is no suggestion in Barbas '201 or the art as a whole to combine different frameworks as set forth in the rejection. Simply put, the skilled artisan would have no reason to believe that altering the recognition helix (as described in Barbas '201) of the only non-canonical finger (the 11th finger) of CTCF and combining this single finger with a different framework would result in a protein with a non-canonical finger that bound to a target site in plant gene.

(e) Combining different zinc finger frameworks was completely unpredictable at the time of filing

The Examiner also errs in asserting that the proposed combination of one of Filippova's fingers into "Barbas '201's polynucleotide" is somehow predictable. *See, e.g.,* Examiner's Answer, page 25.

For the reasons of record and reiterated herein, it is entirely unpredictable from the references and state of the art at the time of filing that altering the single non-canonical finger of Filippova and combining this altered zinc finger with other zinc finger frameworks would result in a functional protein. As set forth by the Supreme Court in *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398; 82 USPQ2d 1385, 1397 (2007) and Patent Office Guidelines regarding determining obviousness issued in view of *KSR*, an obviousness rejection is only proper when the proposed combination of elements results in a predictable outcome (see, Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in view of the Supreme Court Decision in *KSR International Co. v. Teleflex Inc.*, Fed. Reg. Vol. 72, No. 195, October 10, 2007, emphasis added):

> The rationale to support a conclusion that the claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the

> combination would have yielded nothing more than predictable results to one or ordinary skill in the art at the time of the invention.

Rather, the Supreme Court in *KSR* reiterated that an obviousness inquiry is fact-dependent and that "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, 82 UPSQ2d at 1389. The Federal Circuit has consistently reversed a finding of obviousness, even when all claimed elements are individually present in the references. See, e.g., *In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000).

In case on appeal, there is no combination of Barbas '201 and Filippova that establishes that the proteins encoded by the claimed polynucleotides were a predictable use of "known" elements. As admitted by the Office and noted above, Barbas '201 fails to teach or suggest anything about non-canonical zinc finger domains set forth in claims 30 and claims dependent therefrom, namely zinc fingers having 2-4 amino acid residues between the amino terminal zinc coordinating residues and 1, 2, 3, 4, 6 or 7 amino acid residues between the carboxy terminal zinc coordinating residues. Thus, Barbas '201 fails to teach using C3H backbones of the claimed structure.

Furthermore, as reiterated above, Barbas '201 fails to teach, suggest or enable combining different zinc finger frameworks into a single protein. Again, there is not a shred of evidence supporting the Examiner's allegation that isolating a single finger from CTCF, modifying the recognition helix region of that finger and combining it with the polynucleotides encoding a different zinc finger framework (as described in Barbas '201) would predictably result in a functional binding protein in plant cells. To the contrary, the evidence establishes that (1) Barbas teaches away from modifying anything other than the recognition helix region and, more importantly, teaches away from combining different frameworks into a single zinc finger protein; and (2) Filippova teaches away from isolating the framework of finger 11 from the context of CTCF as a whole.

For at least the foregoing reasons, withdrawal of the rejection is in order.

B. The claims are non-obvious over Barbas '728 and Filippova

Claims 25-28, 30-32, 36, 39-41 and 53-57 also remained rejected as allegedly obvious over U.S. Patent No. 7,329,728 (hereinafter "Barbas '728") in view of Filippova, as cited above. (Examiner's Answer, pages 7-10). As with Barbas '201, it was acknowledged that Barbas '728 does not teach isolated polynucleotides encoding a non-canonical zinc finger protein as claimed. However, Filippova was again alleged to teach this element. *Id.*

In response to Appellants' arguments that there is no combination of Barbas '728 and Filippova that teaches the claimed subject matter, it was asserted, as above with regard to Barbas '201, that Barbas '728 teaches the use of any zinc finger framework (including a "completely designed" Sp1 protein) and does "not provide evidence that other frameworks and/or combinations of zinc finger domains would not have predictably bound to their target site." (Examiner's Answer, page 29).

The Examiner's allegations are legally and factually erroneous. In particular, the assertion that the single framework (*e.g.,* Sp1) used in Barbas '728 is "completely designed" (including the framework) is incorrect. The Sp1 proteins of Barbas '728 are designed in the sense that the amino acids making up the recognition helix regions are engineered. There is nothing in Barbas '728 that teaches modifying the rest of the "framework," namely that part of the zinc finger outside the limited area recognition helix region is predictable. To the contrary, Barbas '728 teaches that residues outside the recognition helix region should not be modified (see, Barbas '728, column 42, lines 19-24; col. 45, lines 15-21):

> The framework residues play a role in affinity and specificity. Thus, amino acid positions −2 to 6 of the DNA recognition helices are either grafted into a Zif268 (Pavletich et al. (1991) Science 252:809-817) or an Sp1C framework (Desjarlais et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260).
>
> The framework residues may play a role in affinity and specificity. For helix grafting, amino acid positions −2 to 6 of the DNA recognition helices were either grafted into a Zif268 (Pavletich et al. (1991) Science

> 252:809-817) or an Sp1C framework (Desjarlais et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260).

Furthermore, there is nothing in Barbas '728 regarding combining different frameworks. Indeed, Barbas '728 even teaches away from isolating individual fingers (finger 2 of Zif268 or Sp1) from the same 3-fingered framework and serially connecting them, teaching that such proteins were of limited function (Barbas '728, col. 47, lines 24-26):

> The absence of a substantial increase in the affinity of the E2C(F2) suggested that serial connection of F2 domains is not optimal. ...

It is only when Barbas used the fingers with modified recognition helices in their natural context (*i.e.,* the modified recognition helices of the fingers are in their natural framework) is affinity increased (Barbas '728, lines 30-33):

> In contrast to the F2 domain protein, the E2C(Zif) and E2C(Sp1) six-finger proteins displayed 40- to 70-fold increased affinity as compared to their original three-finger protein constituents.

Therefore, the Examiner errs in asserting that Barbas '728 does not provide evidence that non-natural frameworks, including combinations of single zinc finger domains is unpredictable. Barbas '728 clearly teaches that once a finger is isolated from its natural framework and even when it is recombined with other fingers from the same framework, binding affinity is entirely unpredictable. Certainly, it is entirely unpredictable how single fingers serially connected from different frameworks would function.

Moreover, the Examiner failed to address arguments that her assertion that Filippova teaches that their single non-canonical finger "functions as part of their protein" to bind DNA was clearly in error. (Advisory Action, page 5 and sentence bridging pages 5-6). As noted above, Filippova is unambiguous that finger 11 of CTCF (the only non-canonical finger) is not involved in DNA binding in some instances. Thus,

even if the recognition helix region of this non-canonical finger were altered, it is entirely unpredictable as to whether this individual finger placed in the context of other finger frameworks, would bind to its target site.

In sum, there is no combination of Barbas '728 and Filippova that teaches or suggests isolating Filippova's 11th finger of CTCF, altering the recognition helix of this finger so as to bind to a target site in a plant gene, and combining this finger with other zinc finger frameworks. Moreover, Barbas '728 clearly teaches that serial combination of individual fingers is not predictable. Thus, there is no combination of these references that teaches or suggests the claimed subject matter and the rejection cannot stand.

C. Claim 37 is non-obvious over the cited references

Claim 37 remained rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Barbas '728 in view of Filippova and in further view of Guyer, which was cited as previously for allegedly disclosing GAL4-C1 fusion proteins. (Examiner's Answer, pages 10-12). Barbas '728 and Filippova were cited as above and Guyer was cited for teaching a hybrid transcription factor comprising the DNA binding domain of the *S. cerevisiae* GAL4 protein and the transcription activation domain of the maize C1. *Id.*

For the reasons detailed above, Barbas '728 and Filippova do not teach or suggest, and in fact teach away from, isolating a single zinc finger from its natural framework, altering its recognition helix and combining this single finger with other zinc fingers from different zinc finger protein frameworks. To the contrary, Barbas '728 teaches that framework residues impact both DNA binding affinity and specificity and that binding is in any way predictable only the recognition helix region itself is modified. Guyer fails to cure the deficiencies of Barbas '728 and Filippova.

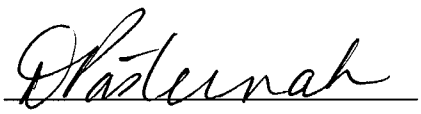
Thus, a *prima facie* case of obviousness has not been and cannot be established and the rejection of claim 37 should be withdrawn.

CONCLUSION

Appellants believe the claims are in condition for allowance and respectfully request that the Board reverse the Examiner and the claims proceed to allowance.

Respectfully submitted,

Date: February 25, 2010

By: 

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CLAIMS APPENDIX

25. The isolated polynucleotide of claim 30, wherein the target sequence is a promoter sequence.

26. The isolated polynucleotide of claim 30, wherein the zinc finger binding protein comprises three zinc finger components.

27. The isolated polynucleotide of claim 30, wherein the target sequence comprises about 9 to about 14 contiguous base pairs.

28. The isolated polynucleotide of claim 26, wherein the third zinc finger component comprises a non-canonical zinc finger component.

30. An isolated polynucleotide encoding a non-naturally-occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein:

(i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine or histidine residues and an alpha helix comprising two carboxy-terminal zinc coordinating cysteine or histidine residues, wherein at least one of the zinc coordinating residues is a histidine residue and at least one of the zinc coordinating residues is a cysteine residue;

(ii) the non-canonical zinc finger component comprises 1, 2, 3, 4, 6 or 7 amino acids between the two carboxy-terminal zinc coordinating residues and 2, 3 or 4 amino acids between the two amino-terminal zinc coordinating residues; and

(iii) the non-canonical zinc-finger binding domain protein comprises a recognition helix of at least 7 amino acids in length, wherein the recognition helix is non-naturally occurring and is engineered to bind to a target nucleic acid sequence in a plant cell.

31. An expression vector comprising the polynucleotide of claim 30.

32. An isolated host cell comprising the polynucleotide of claim 30.

36. The polynucleotide of claim 39, wherein the functional domain is an activation domain.

37. The polynucleotide of claim 36, wherein the activation domain is selected from the group consisting of maize C1, VP16, p65 subunit of NF-kappa B, and VP64.

39. An isolated polynucleotide according to claim 30 further encoding a functional domain.

40. An expression vector comprising the polynucleotide of claim 39.

41. An isolated host cell comprising the polynucleotide of claim 39.

53. A composition comprising a polynucleotide according to claim 39 and a pharmaceutically acceptable excipient.

54. The isolated polynucleotide of claim 26, wherein the first zinc finger component comprises a non-canonical zinc finger component.

55. The isolated polynucleotide of claim 30, wherein the zinc finger binding protein comprises four zinc finger components.

56. An isolated polynucleotide encoding a non-naturally occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein:

(i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine and an alpha helix comprising two carboxy-terminal zinc coordinating cysteine or histidine residues, wherein one of the carboxy-

terminal zinc coordinating residues is a histidine residue and one of the carboxy-terminal zinc coordinating residues is a cysteine residue;

(ii) the non-canonical zinc finger component comprises 2 amino acids between the two amino-terminal zinc coordinating cysteine residues; and

(iii) the protein comprises a non-naturally occurring recognition helix that is engineered to bind to a target nucleic acid sequence.

57. The polynucleotide of claim 56, wherein the carboxy-terminal zinc coordinating histidine residue is amino terminal to the carboxy-terminal zinc coordinating cysteine residue.

EVIDENCE APPENDIX

No documents are attached to this appendix.

RELATATED PROCEEDINGS APPENDIX

As noted on page 2 above, no documents are submitted with this Appendix.